*Original Article*

# Evaluation of menthol per se on acute perceptions and behavioral choice of cigarettes differing in nicotine content

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**Abstract**

Subjective perceptions and self-administration of cigarettes are each influenced by nicotine. Yet, differences specifically due to menthol in perceptions and choice of cigarettes varying in nicotine, and the association between these responses, have not been directly tested. Using a mixed between- and within-subjects design, acute responses to each of two menthol or non-menthol Spectrum research cigarettes, moderate (16–17 mg/g) versus very low (0.4 mg/g) in nicotine contents, were compared following brief abstinence in adult smokers preferring menthol ($n$=44) or non-menthol ($n$=29) brands. To ensure reliable perceptions, they experienced five exposures to each cigarette, then chose between them. All perceptions and choices were greater for moderate vs very low nicotine, as expected, and the magnitude of difference in four of six perceptions was associated with subsequently greater choice of the moderate nicotine cigarette. Importantly, virtually no differences were found between menthol and non-menthol, as nearly all perceptions, cigarette choices, and the association between perceptions and choice were not moderated by menthol or the interaction of nicotine by menthol. Our results indicate perceptions and reinforcement from cigarettes do not differ due to menthol when nicotine content and smoking topography are carefully controlled. Thus, regardless of menthol, smoking perceptions directly predict self-administration behavior.

**Keywords**

Tobacco smoking, menthol, cigarette nicotine content, very low nicotine, subjective perceptions, reinforcement

## Introduction

Cigarette smoking behavior is assumed to be positively reinforced, in large part, by acute pleasurable subjective ("rewarding") effects resulting from the intake of nicotine (Kalman, 2002; Pomerleau and Pomerleau, 1992). Smoking can also be negatively reinforcing when that nicotine intake relieves symptoms of withdrawal after abstaining from tobacco, or perhaps other aversive symptoms (Baker et al., 2004; Eissenberg, 2004). Considerable laboratory-based research has demonstrated greater self-reported positive subjective ratings (Hatsukami et al., 2013; Lindsey et al., 2013; Perkins et al., 2004, 2006) and greater self-administration (Blendy et al., 2005; Higgins et al., 2017a; Perkins et al., 1996, 2002; Ray et al., 2006) when smokers acutely smoke higher vs lower nicotine cigarettes, consistent with the notion that a cigarette's nicotine level increases both its positive subjective and reinforcing effects.

Yet, we know of no direct comparison of acute responses to cigarettes differing in menthol while carefully matched on nicotine content and other constituents. Preclinical research indicates menthol may enhance the reinforcing effects of low-dose nicotine in rats (Biswas et al., 2016), but most menthol-related differences in clinical research are uncertain and stem from surveys, rather than controlled prospective comparison of responses to smoking due to menthol per se (Tobacco Products Scientific Advisory Committee, 2011). Lack of study on acute menthol effects may be surprising, given calls for its regulation by governments (e.g. Bolcic-Jankovic and Biener, 2015; Malone, 2017). However, the response to menthol vs non-menthol cigarettes is not easily disentangled from that of a smoker's menthol vs non-menthol brand preference, which is essentially dichotomous but may be more or less common among certain subpopulations (Kasza et al., 2014; Tobacco Products Scientific Advisory Committee, 2011; Villanti et al., 2016). Thus, matching the menthol content of cigarettes to the menthol preference of participants ensures the observed responses reflect how smokers in the natural environment respond. In contrast, randomizing menthol cigarettes to all can result in potentially irrelevant responses by smokers administered cigarettes with unfamiliar and, by definition, non-preferred flavorings (Strasser et al., 2013).

Moreover, very little well-controlled research actually has demonstrated a direct link between the magnitude of the positive subjective perceptions of a cigarette and self-administration of that cigarette, to confirm this assumed close association between smoked nicotine's subjective and reinforcing effects. One major obstacle to such research has been difficulty controlling nicotine dosing via cigarette smoking. Until recently, subjective responding to different nicotine doses via smoking was assessed using

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commercial brands differing in nicotine "yield" determined by the US Federal Trade Commission (FTC) method, in which a fixed amount of smoke intake by machine estimates the inhaled portions of nicotine (Marian et al., 2009). Yield values were typically engineered by manipulating the ventilation of the cigarette wrapping paper, rather than the tobacco's nicotine content. Since smokers can easily override this ventilation of smoke by covering over the holes (Benowitz et al., 1983; Strasser et al., 2005), the cigarette's labeled yield is an inadequate index of its nicotine dose delivery (St Charles et al., 2010). Thus, variations between how cigarettes are smoked, and not specific differences in their nicotine content, may partly explain variability in the subjective effects and self-administration between cigarettes differing in nicotine yield.

Due to this limited control over nicotine dosing with cigarette smoking, some prior studies explored the association of subjective responses and self-administration behavior by administering nicotine doses via non-smoked methods. For example, dependent smokers who were more likely to choose higher nicotine vs placebo nasal sprays in an ad lib choice session were also those previously reporting greater pleasurable responses (e.g. alert, pleasant, satisfied) to those nicotine vs placebo sprays during initial exposure sessions (Perkins et al., 1997). Very similar results were found with never-smokers administered nicotine doses or placebo via oral capsules (Duke et al., 2015). Also, acute nicotine dosing administered intravenously (IV) indirectly suggests an association, as smokers reported dose-dependent increases in subjective "liking" or "high" at the initial infusions and in the choice of nicotine vs saline infusions over six opportunities later in the session (Sofuoglu et al., 2008). Yet, no direct comparison was reported between these subjective and behavioral responses to IV nicotine. Nevertheless, because the stimuli from cigarette smoking involve more than simply nicotine intake (Addicott et al., 2014; Rose et al., 2010), the relevance of this research on carefully controlled dosing of non-smoked nicotine to smoked nicotine administration is uncertain.

However, now available through the US National Institute on Drug Abuse (NIDA) are research cigarettes engineered to provide specific nicotine contents (i.e. "dose") of the tobacco contained in the cigarette. Most of these research cigarettes, called Spectrum, are matched on non-nicotine constituents, to aid study of effects due to known amounts of nicotine delivery per se via smoking, controlling for the other constituents (Hatsukami et al., 2013; Richter et al., 2016). Critically, this includes parallel versions that are either menthol or non-menthol flavor (see Research cigarettes section below). Thus, with this careful control over nicotine dosing, it is now much easier to conduct tests of differences in subjective effects, as well as self-administration behavior, in response to acute smoking of cigarettes differing in nicotine content as well as menthol. The limited number of controlled studies to date do suggest generally dose-dependent nicotine content effects on positive ratings of "liking," "flavor," "satisfying," "cigarette strength," and estimated amount of nicotine in Spectrum cigarettes (Hatsukami et al., 2013; Higgins et al., 2017a; Perkins et al., 2016). Some recent studies also suggest nicotine dose effects on behavioral choice of Spectrum cigarettes (Higgins et al., 2017a; Perkins et al., 2017). Yet, we are aware of virtually no research that has directly associated the magnitude of subjective ratings with cigarette choice behavior, and no prior study directly comparing these responses to cigarettes matched on nicotine content but differing in menthol. Such research could greatly inform public policies on regulating tobacco products (e.g. Bolcic-Jankovic and Biener, 2015; Tobacco Products Scientific Advisory Committee, 2011).

The current study assessed acute subjective perceptions and subsequent choice behavior of Spectrum research cigarettes differing in moderate (16–17 mg/g) or very low (0.4 mg/g) nicotine contents. Menthol versus non-menthol Spectrum cigarettes equated on nicotine contents were matched to smokers who preferred menthol or non-menthol commercial brands, respectively. We hypothesized a direct association between the magnitude of subjective "perception" ratings and subsequent self-administration behavior of cigarettes differing solely in nicotine contents. Given the lack of prior controlled study of effects on menthol per se, we also explored, but did not hypothesize, possible differences due to menthol.

## Methods

### Participants

Dependent smokers ($n$=73) participated in a single three-hour session, with dependence confirmed by presence of *Diagnostic and Statistical Manual-V* (DSM-V) criteria American Psychiatric Association, 2013) using an updated structured interview (Breslau et al., 1994). Mean (SD) characteristics were age of 33.4 (10.9), 16.4 (5.9) cigs/day, and 5.2 (1.7) score on the Fagerstrom Test of Nicotine Dependence (FTND; Heatherton et al., 1991). No differences due to sex (42 M, 31 F) or menthol preference (44 menthol, 29 non-menthol) were significant for these characteristics, except mean (standard error of the mean (SEM)) FTND was significantly higher for participants who preferred menthol (5.5 (0.2)) compared to those who preferred non-menthol (4.6 (0.3)), $t(71)=2.3$, $p<0.05$. (Consequently, FTND was covaried in comparisons involving menthol; see Data analyses below.) Participants self-identified mostly as Caucasian (69.9%), with 24.7% as African American, and 5.5% more than one race. Those self-identifying as Caucasian were less likely than others to prefer menthol, $X^2(2)=12.86$, $p<0.01$ (also addressed in Data analyses), as commonly reported in the literature (Villanti et al., 2016). One additional male smoker was excluded from analyses due to failure to follow instructions.

### Research cigarettes

Spectrum investigational research cigarettes, manufactured by 22nd Century Group (Clarence, New York, USA; http://www.xxiicentury.com/), were obtained from NIDA's Drug Supply Program. The versions most widely differing in nicotine contents but similar on "tar" yield were selected for study to isolate differences due to nicotine per se. Nicotine contents were approximately 16 mg (menthol) or 17 mg (non-menthol), versus 0.4 mg (for both menthol or non-menthol), per gram of tobacco (i.e. mg/g). (The minimal difference between the higher nicotine content cigarettes was due to the manufacturing process; both were the highest available among the menthol and non-menthol versions matched on tar with its corresponding 0.4 mg version.) All cigarettes had about 9–10 mg "tar". For comparison, the nicotine yields by FTC method are roughly 0.8 mg and 0.03 mg for the two Spectrum research cigarettes (as noted in http://

grants.nih.gov/grants/guide/notice-files/NOT-DA-14-004.html), while US commercial brands typically yield about 0.9 mg nicotine, with 10 mg "tar" (U.S. Department of Health and Human Services, 2010). Because of how these Spectrum cigarettes relate to commercial brands on yield, we refer to them as "moderate" (16–17 mg/g) and "very low" nicotine cigarettes to aid comprehension of the difference.

As noted, to ensure the within-subjects comparison of subjective and behavioral responses to these cigarettes widely differing in nicotine content would reflect how smokers in the natural environment respond to them, participants received Spectrum cigarettes that were matched to their stated commercial cigarette menthol preference. We wanted to control the non-nicotine factors potentially influencing subjective perceptions and choice, including unfamiliar or non-preferred flavorings (such as menthol, e.g. Strasser et al., 2013), so that within-subjects differences between cigarettes were due only to their nicotine contents.

### *Control of cigarette smoking exposure*

Careful control of smoke intake between cigarettes is critical to ensure observed differences in responding are due to their differences in nicotine content per se, rather than to variable amounts of smoke intake (i.e. topography). Smoke intake in this study was standardized at four puffs per trial of exposure to one or the other cigarette, as detailed below in Procedures. In each trial, one puff was taken every 30 s, each with a two-second "hold" duration to target intake of approximately 60 mL per puff, using the portable Clinical Research Support System (CReSS; Borgwaldt KC, Inc., Richmond Virginia, USA). These puffing instructions, designed to simulate topography observed during typical ad lib puffing (Blank et al., 2009; Perkins et al., 2012), were automated and displayed on a computer monitor. Subjects first practiced following these instructions with an unlit cigarette at the start of the session before the trials assessing responses to smoking the Spectrum cigarettes.

### *Measures*

Subjective perceptions of a cigarette's sensory effects were assessed with five self-report items that were found in previous research to be sensitive to cigarette nicotine yield (Perkins et al., 2002, 2006). These five, adapted from Westman et al. (1996) and collectively labeled here "Acute Cigarette Perceptions" (ACP), ask how much "nicotine", "flavor", and "liking" was experienced, and how "satisfying" and "strong" the cigarette was. Items were intended to focus on perceptions likely related to the cigarette's nicotine content, which may explain the cigarette's positive reinforcing efficacy. We specifically avoided items assessing a participant's "mood", such as positive affect or relief of withdrawal symptoms or negative affect, suggesting negative reinforcement. Withdrawal rapidly declines after the first smoking exposure following abstinence (Perkins et al., 2010), precluding a within-session comparison of relief (i.e. negative reinforcement) between different cigarettes. Also assessed was a sixth item asking how "similar to own brand" the cigarette was, to explore whether choice of the moderate nicotine Spectrum cigarette would be greater if it was perceived as more similar to the participant's own brand. Each of these six items was rated on a 0–100 visual analog scale (VAS), anchored by "not at all" to "very much" (Perkins et al., 2012).

Cigarette choice involved subjects being instructed to smoke four puffs, following the automated puffing instructions, from some combination of the two cigarettes made available concurrently, based solely on their preference for each (e.g. all four from one or from the other, or a mix of the two). Of eight total puff choices over the two choice trials, the number of puffs from the moderate nicotine cigarette determined nicotine's relative reinforcing effects (possible range of 0–8). This choice procedure has frequently been used by us and others to evaluate between- and within-subjects factors on nicotine reinforcement (Blendy et al., 2005; Perkins et al, 1996, 2016; Ray et al., 2006).

### *Procedures*

This study was approved by the University of Pittsburgh Institutional Review Board, abiding by the Declaration of Helsinki. The session began by obtaining informed consent and ensuring eligibility. Participants were required to be abstinent overnight from smoking prior to this experimental session. Upon arrival, compliance with these abstinence instructions was confirmed by carbon monoxide (CO)≤10 ppm (SRNT Subcommittee on Biochemical Verification, 2002) via BreathCO CO monitor (Vitalograph, Lenexa, Kansas, USA).

Then, the "moderate" and "very low" nicotine Spectrum cigarettes were intermittently presented, one per trial in random order across 10 trials (five per cigarette), for rating of subjective perceptions of each cigarette. Participants were instructed that two different cigarettes would be evaluated but kept blind as to the nicotine content of each one administered, both of which had no identifying labels on the paper and were thus identical in appearance. All trials consisted of four puffs (about one-third of a full cigarette, as in Hatsukami et al., 2013), separated by 15 min, and so total exposure over the three-hour session was intentionally no more than that from ad lib smoking in the morning after overnight abstinence (e.g. Hatsukami et al., 1988). Note that half the exposures here involved very low nicotine intake, further minimizing chances of nicotine toxicity or satiation, as in similar research comparing responses between cigarettes within a session (Perkins et al., 2002, 2017). After the four puffs in each of these 10 trials, participants completed the brief ACP measure on their subjective perceptions of that cigarette (see Measures section of Methods, above). To help participants differentiate the two in preparation for the subsequent choice trials assessing their relative reinforcement, the moderate and very low nicotine cigarettes were given letter codes of "A" and "B" in the first four trials of exposure (two for each cigarette), with codes randomly assigned to the moderate or very low nicotine cigarette. Following the subjective perception trials, testing each cigarette separately one per trial, participants then engaged in the two choice trials, involving behavioral choice of puffs between the two cigarettes now made available concurrently (and again labeled "A" and "B").

### *Data analyses*

Subjective perceptions of the cigarettes were averaged across the perception trials (five for each cigarette), with the mean of these five items used as the composite ACP measure. As noted earlier, all analyses that included menthol as a between-subjects factor controlled for FTND scores. Initial analyses compared perception between cigarettes using a repeated measures multivariate

**Table 1.** Mean (standard error of the mean (SEM)) acute cigarette perceptions by nicotine content and menthol (non-menthol *n*=29, menthol *n*=44). The first five comprise the Acute Cigarette Perceptions (ACP) scale.

| | Moderate nicotine (Mod) | | Very low nicotine (VLN) | | *Difference* (Mod-VLN) |
|---|---|---|---|---|---|
| | Non-menthol | Menthol | Non-menthol | Menthol | |
| Liking | 56.3 (3.6) | 55.6 (3.0) | 31.6 (3.9) | 33.3 (3.1) | 23.3 (2.7)[a] |
| Satisfying | 57.7 (3.5) | 55.8 (2.8) | 30.8 (3.7) | 32.9 (3.0) | 24.5 (2.7)[a] |
| How much nicotine | 60.1 (3.2) | 58.3 (2.6) | 32.5 (3.2) | 34.6 (2.6) | 25.2 (2.7)[a] |
| Strong[b] | 52.2 (3.4) | 56.4 (2.5) | 25.9 (3.3) | 38.7 (2.7) | 21.1 (2.8)[a] |
| Flavor | 52.4 (3.1) | 53.6 (2.5) | 33.5 (3.8) | 37.8 (3.1) | 17.0 (2.5)[a] |
| Similar to own brand | 45.3 (4.2) | 39.1 (3.5) | 19.8 (2.7) | 19.1 (2.8) | 22.2 (2.9)[a] |

Note. [a]$p<0.001$ for main effect of nicotine content; [b]$p<0.01$ for main effect of menthol.

analysis of variance (RM MANOVA), with cigarette nicotine content (moderate/very low) as a within-subjects factor, and menthol/non-menthol as a between-subjects factor. Univariate follow up RM ANOVA identified which items varied significantly between cigarette types. A Poisson generalized estimating equation was used to confirm differences in number of choices (i.e. count data) due to cigarette nicotine content.

After confirming equal smoking exposure between administered cigarettes, we separately evaluated differences in perceptions and in choice due to cigarettes differing in nicotine content and in menthol. (Because menthol preference differed by ethnicity, as noted in Participants, we repeated the analyses on cigarette perception and on choice involving the between-subjects menthol factor for only the 51 Caucasian participants, and we include those findings in the relevant Results sections.) The main analyses of interest related responses between the two measures, i.e. the association of the magnitude of subjective perceptions to the number of moderate nicotine puff choices. Difference scores between cigarettes (moderate nicotine – very low nicotine) were computed for the ACP composite, and the five perception items individually, to isolate the effect of nicotine separate from smoking behavior per se. Poisson regression was used to model the association between the composite ACP difference score and the number of moderate nicotine cigarette puffs in the subsequent choice procedure. To determine which of the perception items contributed to this significant association, similar Poisson regressions were run on the difference scores for each individual item. One additional Poisson regression was run to model the relationship between the "similar to own brand" item and number of moderate nicotine cigarette puff choices. All analyses were performed in IBM SPSS 24.

## Results

### *Smoking exposure*

Preliminary analyses compared smoking topography per four-puff trial in all subjects between the moderate and very low nicotine cigarettes, and between the menthol and non-menthol subgroups (while also controlling for FTND), to confirm similar exposure to each. As intended, no differences in smoking topography were found, for menthol and the menthol×nicotine content interaction, $F(1,70)$'s of 2.55 and 0.06, both $p$'s>0.10, respectively. The main effect of nicotine content also was not significant, $F(1,70)=0.87$, $p=0.35$, with adjusted means (SEM) for four puffs per exposure of 260 (9) versus 253 (8) mL for the moderate versus very low nicotine cigarettes, respectively. These adjusted mean volumes correspond to 65.0 versus 63.3 ml per puff, consistent with the 60 mL per puff intended by the automated puffing instructions (see Methods).

### *Perception ratings*

The means for the subjective perception items rated on each cigarette are presented in Table 1. As expected, the multivariate analysis indicated a significant main effect of cigarette nicotine content across the linear combination of the subjective responses, $F(6,67)=15.34$, $p<0.001$. Follow-up univariate analyses showed each of the subjective perception items differed significantly between the moderate vs very low nicotine cigarettes, all $F(1,72)$'s>45.00, $p$'s<0.001. Secondary analysis of the subjective responses, including menthol as a between-subjects factor and FTND as a covariate, found a main effect of menthol $F(5,66)=2.84$, $p=0.02$. Very importantly however, the nicotine content×menthol interaction was not significant, $F(5,66)=0.7$, $p=0.66$, indicating the magnitude of perceptions due to the cigarette's nicotine content did not vary by menthol. (After repeating these menthol analyses for Caucasian participants only, results were unchanged for the main effect of menthol, $F(5,44)=2.57$, $p=0.04$, and for the nicotine×menthol interaction, $F(5,44)=1.75$, $p=0.14$.) Follow-up univariate analyses indicated a main effect of menthol for only one of the five ACP items, "how strong," $F(1,70)=9.83$, $p=0.003$, with mean (SEM) ratings of 48.0 (1.9) vs 38.5 (2.3) for menthol vs non-menthol, respectively, collapsed across the moderate and very low nicotine cigarettes for each flavor (see also Table 1).

### *Cigarette choice*

Very similarly, participants chose significantly more puffs from the moderate nicotine versus the very low nicotine cigarette, Wald $\chi^2(1)=31.40$, $p<0.001$, odds ratio (OR)=2.016, 95% confidence interval (CI) (1.678–2.421), also as expected, with no significant differences due to menthol or the menthol×nicotine interaction, both Wald $\chi^2(1)<1$, $p>0.40$. (Again, repeating these puff choice analyses involving the menthol factor for only Caucasians, results were unchanged due to menthol and the menthol×nicotine interaction, both Wald $\chi^2(1)<1$, $p>0.50$.) The

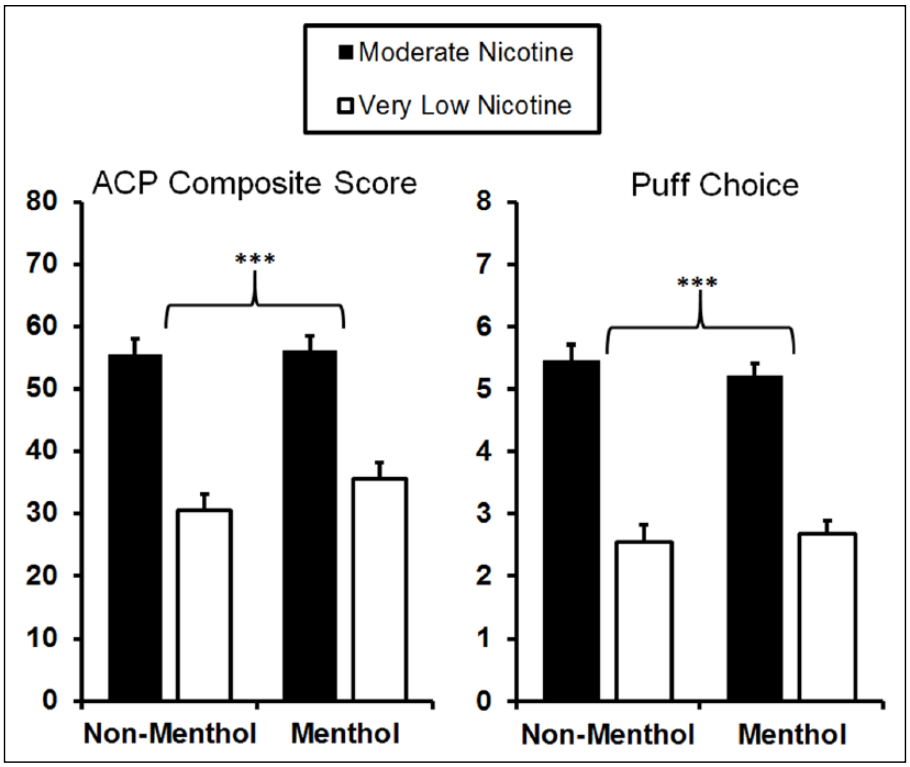


**Figure 1.** Adjusted mean (standard error of the mean (SEM)) for Acute Cigarette Perceptions (ACP) scale score and choice of puffs from Spectrum cigarettes moderate vs very low in nicotine content (controlling for Fagerstrom Test of Nicotine Dependence (FTND) scores). Bars are shown separately for non-menthol ($n$=29) and menthol ($n$=44). Effects on both measures were highly significant for differences in cigarette nicotine content ($p$'s<0.001) but not menthol vs non-menthol ($p$'s>0.29). ***$p$<0.001 for main effect of nicotine content.

means for the composite ACP score (comprising the five items in Table 1) and for cigarette choice are shown in Figure 1, separately by moderate and very low nicotine content, as well as by menthol/non-menthol.

### *Association of differences in cigarette perceptions with choice*

Since no effects were seen for menthol on cigarette choice, we combined results for the menthol and non-menthol subgroups to examine the association of perceptions with choice in all 73 participants. As shown in Figure 2, there was a significant positive association between the difference in composite ACP score and choice of the moderate nicotine cigarette, Wald $\chi^2(1)=5.64$, $p<0.05$, OR=1.006, 95% CI (1.001–1.011). An OR of 1.006 essentially indicates that, for every 10-unit increase in the perception rating difference between the moderate vs very low nicotine cigarette, the odds of choosing the moderate nicotine cigarette puff was increased by 6%. Although modest, the magnitude of the OR is dependent on the scale of the predictor (Menard, 2010), which ranged from −22.56 to 76.28 (out of a maximum range for VAS difference between cigarettes of −100 to 100). (To confirm no effects due to menthol, we repeated this analysis to include menthol vs non-menthol as a between-subjects factor and FTND as a covariate, finding neither a main effect, Wald $\chi^2(1)=0.96$, $p=0.33$, nor interaction effect, Wald $\chi^2(1)=0.75$, $p=0.39$, on the number of moderate nicotine cigarette choices.)

Very similar associations were observed in the follow-up examinations of each individual item from the ACP scale, as well as with the separate "similar to own brand" item (not shown). Three of the five subjective perception item difference scores had significant positive associations with number of puff choices, "liking" (Wald $\chi^2(1)=6.36$, $p<0.05$, OR=1.006, 95% CI (1.001–1.010)), "satisfying" (Wald $\chi^2(1)=6.63$, $p<0.05$, OR=1.006, 95% CI (1.001–1.010)), and "how much flavor" (Wald $\chi^2(1)=4.98$, $p<0.05$, OR=1.005, 95% CI (1.001–1.010)). "How much nicotine" was marginally associated with number of puff choices, Wald $\chi^2(1)=3.74$, $p=0.05$, OR=1.004, 95% CI (1.000–1.009), but "strong" had no significant association, Wald $\chi^2(1)=1.16$, ns, OR=1.002, 95% CI (0.998–1.006). Separately, "similar to own brand" was also significantly positively associated with the number of puff choices, Wald $\chi^2(1)=3.88$, $p<0.05$, OR=1.004, 95% CI (1.000–1.010).

## Discussion

Acute subjective perceptions and choice behavior differed significantly between research cigarettes that were moderate vs very low in nicotine content. These findings were expected based on studies outlined in the Introduction, which separately compared cigarettes differing in nicotine on perceptions or on self-administration. The current results go beyond that prior research in several ways, including our very careful control over the content of nicotine exposure from cigarette smoking, the direct comparisons of

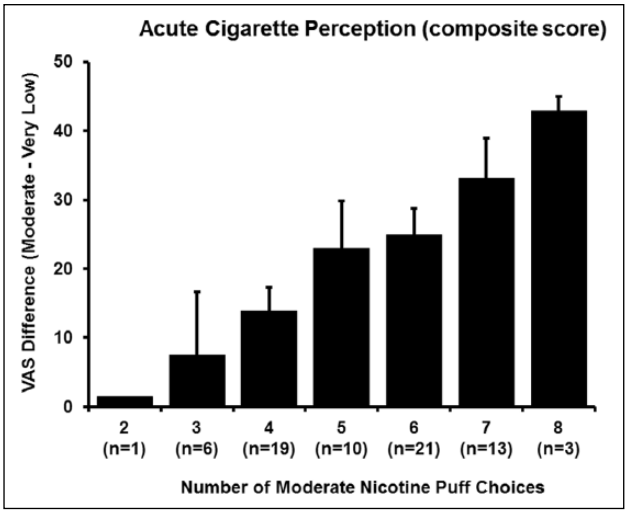


**Figure 2.** Association of Acute Cigarette Perceptions (ACP) scale score with choice behavior as a function of differences between cigarettes moderate vs very low in nicotine content. Shown are mean (standard error of the mean (SEM)) differences between cigarettes in composite score on the ACP (comprising five items), by the subsequent number of choices for the moderate nicotine cigarette (possible range of 0–8) in all participants ($n$=73). This association ($p$<0.05) indicates the greater the difference in ACP score, the greater the relative reinforcing effects of smoked nicotine.

responses due to menthol per se, and associating the magnitude of perceptions with choice behavior. Regarding the last point, our findings clearly indicate that the greater the difference in acute subjective perceptions between cigarettes varying in nicotine content, the greater the choice of the higher nicotine cigarette. Thus, acute reinforcing effects of nicotine from smoking a cigarette are directly and linearly associated with these subjective perceptions of that cigarette. This association may seem an obvious one but, as noted, very little research had systematically tested this notion to help clarify acute effects of nicotine that may explain behavior reinforced by smoking cigarettes.

Sharply in contrast, aside from a main effect of menthol on one of five ACP items ("how strong"), there were no differences in either perceptions or choice, or in their association, between menthol and non-menthol cigarettes (or the nicotine×menthol interaction). Indeed, virtually identical responses to these cigarettes between menthol and non-menthol versions (Table 1, Figure 1), controlling for nicotine, confirm the very close matching of nicotine contents between these flavors of Spectrum cigarettes. More importantly, such results also demonstrate that, under the conditions of this study, few effects of menthol per se (or as a function of menthol preference) are observed in adult smokers when the nicotine content and smoking topography of administered cigarettes are very carefully controlled. Lack of prior controlled comparisons in acute responding between cigarettes differing only in menthol renders our study results a very novel contribution to the smoking literature. Given controversies over the role of menthol in reinforcing smoking behavior, especially in teens or other subpopulations (Tobacco Products Scientific Advisory Committee, 2011), replication of these findings is warranted. These menthol and non-menthol Spectrum cigarettes, matched carefully on nicotine content (and tar), may provide a very effective tool for further tests of smoking responses due to menthol per se.

Among the implications of our results, differences in perception ratings and choice behavior should be smaller in response to cigarettes differing less markedly in nicotine content, and the association between perception and choice of cigarettes may be comparably attenuated, relative to the moderate vs very low nicotine cigarettes here (Perkins et al., 2017). The current study intentionally compared those Spectrum cigarettes differing most widely in nicotine content while similar in "tar" and matched on menthol. A second implication is that individual differences in sensitivity to perceiving cigarettes varying in nicotine content may also help explain individual differences in smoking reinforcement, as indicated in Figure 2. As examples, systematic differences in perception and choice may improve our understanding of variations in: nicotine dependence onset and prevalence (Strong et al., 2012), preference for cigarettes higher or lower in nicotine (Higgins et al., 2017b), difficulty initiating or maintaining abstinence (Goodwin et al., 2011), or other typical smoking patterns (e.g. Bondy et al., 2013). Third, this association between greater perceptions and choice behavior could apply to other nicotine-containing products, such as electronic cigarettes, suggesting those products may also be reinforcing due to similarly greater subjective perceptions from acute use (Audrain-McGovern et al., 2016; Dawkins and Corcoran, 2014). Such products may also show that other flavorings do, or do not, alter this association (Audrain-McGovern et al., 2016; Rosbrook and Green, 2016). Finally, further research on neural and behavioral mechanisms responsible for the subjective perception and reinforcing effects of smoked nicotine (Brody et al., 2009; Russell, 1989) may benefit from testing these responses concurrently as in this study, rather than separately as in most prior studies. Such an approach could be relevant for addressing the distinctions between "liking" (e.g. subjective perceptions) and "wanting" (i.e. self-administration) of cigarettes differing in nicotine content (Berridge and Robinson, 2016).

Future research could also focus on why all the mean perception ratings (perhaps except "similar to own brand", not an ACP item) were well above zero for the very low nicotine cigarette (Table 1), even for "how much nicotine". We did not have a true "placebo" cigarette devoid of any nicotine for comparison, but why just four puffs on the very low 0.4 mg/g nicotine cigarette would elicit ratings on "liking", "satisfying", etc. that were one-third of the maximum possible rating (i.e. around 33 on 0–100 VAS), compared to just over half for the moderate nicotine cigarette, seems worthy of study. These observations appear consistent with other research demonstrating substantial perception, self-administration, and other responses to denicotinized cigarettes (Brody et al., 2009; Donny et al., 2006; Rose et al., 2010), supporting the contributions of very low amounts of nicotine exposure, or of non-nicotine (and possibly secondary reinforcing) factors, to smoking perceptions or behavior (e.g. Donny et al. 2006; Perkins et al. 2004).

Regarding potential limitations of this study, the strength of the associations seen here may depend on the specific procedures used. This study necessarily controlled for all non-nicotine factors that could alter responding, so that just nicotine content of cigarettes would differ. We did so using a within-session comparison of cigarette perceptions as well as of

subsequent cigarette choice behavior, which potentially could introduce carry-over effects. Yet, that was why exposure to each cigarette on each trial was limited to four controlled puffs, to avoid smoke toxicity or satiation across the half of all trials in which the moderate nicotine cigarette was administered. We have not observed carry-over effects of this pattern and timing of controlled smoking of different cigarettes following overnight abstinence (Perkins et al., 2017). We also chose four puffs per exposure based on prior research with Spectrum cigarettes (Hatsukami et al., 2013), and the fact that subjective ratings of cigarettes are often formed within the first few puffs (Hasenfratz et al., 1993). Furthermore, our prior research suggests concurrent testing of different cigarettes may enhance, not attenuate (as with carry-over effects), the magnitude of difference in responses between cigarettes (Perkins et al., 2002). Also, a lack of Bayesian analyses to evaluate the level of support for the observed null effects of menthol can be regarded as a limitation. Finally, the association of perceptions with cigarette choice observed here may be specific to smokers who are nicotine dependent, as non-dependent smokers may have similar perceptions but less choice of the moderate nicotine cigarette. Such a finding would be consistent with their lack of dependence on nicotine reinforcement (virtually by definition) and comparable to our prior test of nicotine discrimination threshold and choice between groups due to their presence vs absence of dependence (Perkins et al., 2017).

In conclusion, perceptions and choice of cigarettes varying in menthol per se (or in those preferring menthol vs non-menthol brands) do not differ when those cigarettes are carefully matched on nicotine content and puffing topography. Therefore, regardless of menthol flavoring, the magnitude of difference in acute increases in subjective perceptions between cigarettes differing in nicotine content is directly associated with a smoker's subsequent choice behavior between cigarettes. Our results indicate that smoking reinforcement is closely linked to the subjective positive perceptions of a cigarette, whether a menthol or non-menthol brand. Individual differences and other factors influencing variability in these acute perceptions and choice behavior, perhaps as well as the association between them, may improve understanding of smoking preferences, patterns, and motivations (Russell, 1989).

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